Results: FGF9 had significant beneficial effects on multiple parameters used to assess cartilage damage. FGF9 treatment reduced the cartilage degeneration score for the outer region of the medial tibial plateau by 33% ($p = 0.004$), the width of significant cartilage damage by 38% ($p = 0.018$), and the depth of cartilage lesions by 20-44% ($p = 0.003$). Image analysis showed that FGF9 increased the total cartilage area by 24% ($p < 0.001$) and the viable cartilage area by 35% ($p < 0.001$). Proteoglycan loss was reduced by 43% ($p = 0.003$) and the area that showed minimal damage to the collagen was increased two-fold. FGF9 had no significant effect on the subchondral bone but increased the size of the chondrophytes/osteophytes by 29% ($p = 0.001$).
Conclusions: The local delivery of FGF9 in an OA model provided significant beneficial effect on the damaged cartilage. Our data indicates that FGF9 may be a disease-modifying drug candidate for the treatment of osteoarthritis.
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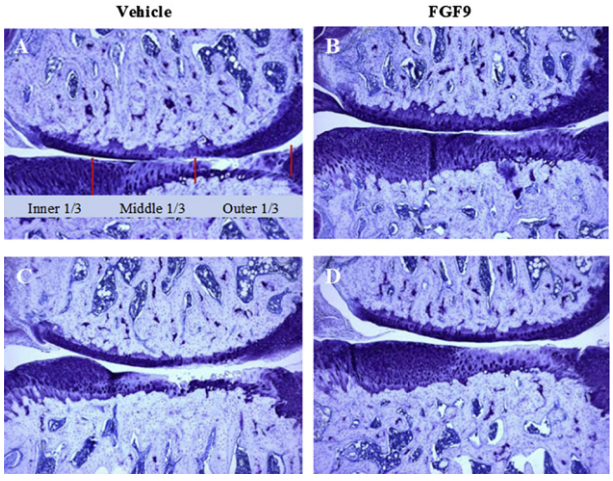

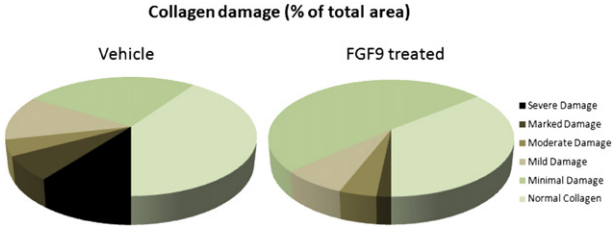

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INCREASED METHYLATION STATUS OF SOX9 GENE PROMOTER IN HUMAN OSTEOARTHRITIC CARTILAGE

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Purpose: DNA methylation and modification of nucleosome histone tails are essential contributors to the mechanisms of epigenetic control of gene expression. In this study, we tested the hypothesis that methylation status of SOX-9 gene promoter region is different in osteoarthritic (OA) cartilage from those of normal cartilage.
Methods: Femoral heads were obtained from 10 patients who had femoral neck fractures without OA, and also from 10 OA patients undergoing total hip arthroplasty. For methylation specific polymerase chain reaction (MS-PCR), CpG rich regions within upstream sequences 5 kb from the transcription start site were analyzed. Putative CpG-rich islands and respective primers for MS-PCR were searched from the CpG Island Searcher and the MethPrimer program, respectively. In bisulfite sequencing, primers were designed based on MethPrimer program and sequences were analyzed using 2BLAST.
Results: Methylation of SOX-9 promoter region increased in OA cartilage compared to normal cartilage. From MS-PCR, methylation status of SOX-9 for R3 (from -3653 to -3496, p=0.0186) and R4-1 (from -3111 to -2983, p=0.0014) significantly increased in OA cartilage compared to normal cartilage. When we analyzed regions (BSQ1-5) from -4548 to -2846 in the promoter of SOX-9 by bisulfite sequencing, methylated CpG sites significantly increased in all the examined regions: total methylated CpG sites increased about eight-fold in OA cartilage (14.04%) than in normal cartilage (1.66%).
Conclusions: Our study suggests that the increased methylation status in the SOX-9 promoter region may have a close relation to the progression of OA.

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MICROARRAY STUDIES OF SYNOVIAL SPECIMEN OF EARLY HUMAN (CHECK) AND EXPERIMENTAL OA IDENTIFY PATHWAYS AND PROCESSES ASSOCIATED WITH CARTILAGE DAMAGE

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Purpose: Over 50% of osteoarthritis (OA) patients show synovial inflammation, even relatively early during the disease. However, the mechanisms through which this synovial activation contributes to the irreversible joint pathology that characterizes OA, are not known. In the present study we used microarray analysis of synovial tissue of early OA patients and of experimental OA, to identify common pathways that determine cartilage damage in this disease.
Methods: From a subpopulation of patients that entered the CHECK Cohort study (Cohort Hip and Cohort Knee), synovial biopsies were collected. CHECK is a prospective 10-year follow-up study that was initiated by the Dutch Arthritis Association on participants with early osteoarthritis-related complaints of hip and/or knee. Radiographs are taken in a standardized manner and scored (Kellgren&Lawrence KL) at inclusion (n=18). In addition, biopsies of 7 control synovia were collected. A longitudinal expression analysis was performed on murine synovial tissue at day 7, day 21 and day 42 after induction of collagenase induced OA (CIOA). CIOA was induced by intra-articular injection of collagenase, which causes joint instability, and contra lateral knee joints served as controls. Initial analysis of microarray data was performed using Partek software and functional annotation clustering (FAC) and pathway analysis was done using DAVID.
Results: Gene expression profiles of control synovia were compared to CHECK synovia. Analysis using DAVID indicated enrichment of several biological processes and signaling pathways, including regulation of macrophage differentiation, innate immune responses, cell migration, TGFβ-, BMP- and wnt-signaling. This indicates clear activation of the synovium in the CHECK patients compared to controls. Next we compared synovial tissue of CHECK-patients with radiological damage (KL≥1) with CHECK-patients without joint damage (KL=0). Among the top 30 genes that were strongest associated with cartilage damage were MMP-1 (18-fold), MMP-3 (10-fold), S100A8 (6-fold) and cartilage glycoprotein-39 (6-fold), all of which have been associated with cartilage damage. Immunohistochemical staining revealed that expression of MMP-1 and MMP-3 was highest in the synovial lining layer. FAC analysis showed that, among others, response to wounding, chemotaxis, innate immune response and metalloproteases were strongly and significantly enriched and thus associated with joint damage. Pathway analysis demonstrated that in the synovium of patients with joint damage the complement-activation pathway, TGFβ- and BMP-signaling and TLR-activation were significantly upregulated. These results were further underlined by analysis of synovium from experimental OA. Among the genes that were strongly upregulated on all 3 time points after induction were MMP-3 (6-fold), MMP-13 (16-fold), MMP-14 (6-fold) and COMP (13-fold). Again, wound healing, innate immune response and metalloproteases were significantly enriched, as were the complement pathway, the TLR-, TGFβ, BMP and wnt-signaling pathways. In a recent publication, complement was demonstrated to be essential in experimental OA. We therefore determined whether